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# SOME COMPARISONS OF BACTERIAL PLANT GALLS AND OF THEIR CAUSAL AGENTS[1]

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## INTRODUCTION

Pathological growths on plants arise from stimulation by various agencies, and many of them are so harmful that they are commonly classed as diseases. Among the agents causing harmful galls are insects, nematodes, fungi, bacteria and various non-parasitic factors. In the present paper the discussion is limited to pathological growths caused by pathogenic bacteria and to certain similar overgrowths caused by non-parasitic agencies. Consideration is omitted both of the physiology of crown gall proper, because comparable studies have not been made of other galls, and of legume root nodules, because they are beneficial (*cf.* 20).

Bacterial galls have been known to plant workers for many centuries. The causal relation of bacteria, however, was not clearly established until Erwin F. Smith and his co-workers published their classic early work on crown gall (80, 81). This is the best known of the bacterial galls. Since that time various other gall-inducing micro-organisms have been described from different localities.

The geographic region where these bacteria first appeared is by no means certain. Doubtless they have been spread all over the world as a result of plant shipments, an obvious means of dissemination. Some investigators (*e.g.*, 7) have considered that crown gall,

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Since this manuscript was prepared, a virus that causes growth has been described: L. M. Black, A virus tumor disease of plants. Am. Jour. Bot. 32: 408–415. 1945.

at least, because of its natural occurrence in isolated cactus forests, was indigenous to the Southwest, and others, including the present senior writer, have observed crown gall on susceptible hosts in other areas which were never cultivated.

The damage caused by these galls is greatest on susceptible crops which have been grown intensively in the same field. At one time piece-root-grafted nursery apple trees had graft knots (including crown gall, hairy root and wound overgrowths) to such an extent that a third of the susceptible trees had to be destroyed. Repetition of such loss has been prevented by improved methods of making and wrapping the grafts (*e.g.*, 49, 64, 65, 66). Crown gall on sugar beets has been quite destructive (92) unless at least a two-year rotation was employed. It is still a problem in widely scattered areas on stone fruit trees.[2] On cane fruits crown gall may be serious (1), but cane gall (29) is much less damaging. The two may be easily confused. Olive knot has been quite harmful on certain varieties of olive (98). An estimate of the economic importance of these and of other galls may be found in the papers in which the bacteria are described.

Important as these galls are on agricultural crops, a study of them seems to have value also from the standpoint of the basic phenomena involved in pathological cell growth. The fundamental problem, whether in plants or animals, has been well expressed by Szent-Györgyi (90) as follows:

"Biochemistry teaches us that many constituents of our body are found with equal frequency in plants and animals, fulfilling analogous functions in each. These substances of plants, just as they are, or with little alteration, fit into the machinery of our cells. Two machines, the parts of which are interchangeable, cannot be very different and so anything we learn about the plant will lead us closer to the understanding of ourselves. The plant, as an object of the study of life, has, compared to man, some very great advantages. . . . The plant . . . can dispense with many of the unessential complications found in our body which enable us to walk, hear, see, smell, and think. Life in the plant will present itself in much simpler forms, and thus allow the great fundamental principles to come to the fore".

Some of the advantages provided by plants for fundamental studies in connection with galls may be listed (*cf.* 58) as follows:

(*a*) Large numbers are easily available. The number used, whether ten, a hundred or a thousand, is commonly adjusted to the statistical needs of an experiment.

[2] Since this manuscript was prepared, J. G. Brown (Science **100**: 528. 1944) has reported the control of crown gall with a penicillin preparation. Among the chemical treatments of galls, those suggested by P. A. Ark (Blue Anchor **19**(1): 16–19. 1942) appear promising.

(*b*) The initial cost and the expense of maintaining plants are very low in comparison with those of animals.

(*c*) The species of plants attacked frequently contain varieties or selections possessing several degrees of resistance and susceptibility.

(*d*) Plants are suited to a wide range of experimental procedures, many of which are not feasible with animals.

(*e*) Epidemics caused by micro-organisms are induced with relative ease and can be studied without concern for the health of the technicians or the public. Likewise, non-parasitic but pathological growths occur spontaneously or as a result of physiological disturbances.

(*f*) The genetic purity of the host can be assured. Seed from long lines of successively self-fertilized parents are already available in many varieties of plants. When this is not sufficient, one can commonly find, or develop, experimental units all genetically identical through vegetative propagation. For example, within a named variety of many fruits and ornamentals, the numerous individuals are all vegetative parts of one originally selected parent. Except for occasional bud variations, they are all genetically the same. This is particularly advantageous in studies of disease and resistance, since pathogenicity is necessarily defined in terms of susceptibility of the host.

(*g*) Plant tissue can be cultivated *in vitro* on a medium containing only constituents of known chemical composition (reviewed, 95). This enables the plant worker to make various studies with accurate controls that to date are not possible with animal tissues.

The obvious possibilities with plant materials led Dr. James Ewing (personal communication), the well known cancer pathologist, to suggest in 1908 to Erwin F. Smith that a fundamental study of pathological growth be made with crown gall. The possible contribution to the cancer problem first inspired Smith to a long series of well known and monumental works, and subsequently stimulated a large number of other workers all over the world.

Smith did not hesitate to use various medical terms and described his different kinds of crown gall as one or another type of malignant tumor or cancer. This attracted much attention and support to his work. Various medical men praised him, while others, including Dr. Ewing, criticized him, saying that his terminology was

inaccurate and therefore misleading and distracting from the real problem. Smith's usage of medical terms has been more or less followed by various more recent workers (*e.g.*, 40, 96) with rather similar praise and criticism. The crux of the matter seems to center about the definition of a cancer, a term which is clear enough in many medical cases, but which is difficult to delimit. Thus, what is or is not a cancer depends on the expert consulted. But, after all, the physiological mechanism of a pathological growth is not changed, whether it is called by one name or another. The real importance attaches to accurate experiments and clear thinking about their interpretation so as to understand these pathological cells.

Medical terms have been studiously avoided in this paper with the hope of evading the popular reports that follow any research with a connection to cancer, of avoiding fruitless discussions over definitions, of clarifying without glamour the comparisons of different kinds of bacterial plant galls as well as their causal agents, and of assisting readers unfamiliar with the problem and its implications to an accurate comprehension of the situation.

Much of the earlier literature on crown gall has been already reviewed (62), and the relations between pathological growths in plants and animals have been discussed in many well known works (*e.g.*, 40, 43, 53, 79).

In the present paper we have tried to compare the different kinds of bacterial galls as well as their causal agents and to amplify any well defined characters in which either galls or bacteria are distinctly different or prominently similar. Such a study has promise because any similarity between their biochemistry and physiology might indicate factors important in the ultimate causal relation between the pathogen and the host. Likewise, variation in character might indicate that such characters have less promise of a causal relation. While such a consideration may be based on the concept that the fundamental stimulus is more or less similar in these different types of galls, it does not necessarily follow that this is the true situation. However, it seems obvious such working hypotheses have value. We have tried also to compare with the bacterial gall some similar but non-parasitic growths that may assist in clarifying some of the factors involved.

Concerning the physiology of crown gall and of crown-gall

bacteria, there is an extensive literature covered by earlier reviews (40, 62). However, so relatively little is known about the physiology of the other bacterial galls, that the physiology of crown gall has been reserved for later consideration elsewhere. Occasional important papers have doubtless been missed, and many of the less important or less representative citations have deliberately been omitted.

## DIFFERENT KINDS OF BACTERIAL GALLS

The more prominent bacterial growths, together with their causal agents and more common hosts, are listed in Table 1. The wide host range of the crown-gall bacteria is continually growing beyond our former understanding of it (45). Out of 101 species tested belonging to 32 families, only 21 species failed to show infection. Montemartini's (48) list of hosts is perhaps the most complete. Doubtless it could be enlarged by improved inoculation technique on other hosts. The senior writer (unpublished) once inoculated many local Wisconsin weeds and secured galls on practically all the dicots except those having strongly acid sap. It is striking that the crown-gall organism has a wide host range, while those of

TABLE 1

SUMMARY OF BACTERIAL GALL DISEASES, CAUSAL AGENTS AND HOST PLANTS

| Disease | Causal organism | Prominent hosts |
|---|---|---|
| Beet pocket rot | *Phytomonas beticola* (Smith, Brown, and Townsend) Bergey *et al.* | Sugar and garden beet varieties, (9) |
| Cane gall | *Phytomonas rubi* Hildebrand | Raspberries and blackberries, (29) |
| Crown gall | *Phytomonas tumefaciens* (Sm. & Town.) Bergey *et al.* | Very wide range, esp. on Rosaceous species, (45, 48) |
| Douglas fir gall | *Phytomonas pseudotsugae* (Hansen and Smith) Bergey *et al.* | Douglas spruce, (24) |
| Gypsophila gall | *Phytomonas gypsophilae* (Brown) Magrou | Baby's breath, (10) |
| Hairy root | *Phytomonas rhizogenes* Riker *et al.* | Apple, rose, (61) |
| Oleander knot | *Phytomonas tonelliana* (Ferraris) Adams and Pugsley | Oleander and olive, (75) |
| Olive knot | *Phytomonas savastanoi* (E. F. S.) Bergey *et al.* | Olive and ash, (73) |
| Pea fasciation | *Phytomonas fasciens* Tilford | Sweet pea, garden pea, petunia, geranium, tobacco, (91) |

other gall-inducing bacteria are quite limited. This suggests a relatively broad base for the biological phenomena which are responsible for crown-gall development.

Resistance to crown gall is well known. Different kinds of Malling apple root stock have shown (25) characters from susceptible (No. II) to highly resistant (No. XVI). Similar resistance in *Prunus* stock has been determined (72, 74).

Host specificity for some strains of crown gall has been indicated. For example, crown-gall bacteria were isolated from naturally occurring galls on asparagus, bean and various other plants, and all the cultures obtained were pathogenic over a wide range, except that from bean, which infected only bean and none of the other plants, including *Datura, Pelargonium, Solanum* and *Helianthus* (83, 84, 86). English cultures (100) have varied in their ability to infect certain hosts. Host specificity in one strain from hops and another from walnut has also been found (47). These strains were actively pathogenic on tomato, sunflower and *Bryophyllum,* for example, but produced no or only a slightly pathogenic reaction on Paris daisy.

The comparative pathogenicities of *Phytomonas tumefaciens, P. beticola, P. savastanoi, P. tonelliana* and *P. rubi* were examined by cross inoculations into their respective hosts. Every organism, except *P. tumefaciens* which induced galls on all species used (52), was pathogenic on its own host but was non-pathogenic on the principal host of the other organisms.

### COMPARISON OF BACTERIOLOGICAL CHARACTERS

Some characters of the various bacteria are compared in Table 2, according to Elliott's (19) procedure. Some reservations are necessary about these comparisons. Pinckard's work (52) was the only instance found in which a group of the organisms was used simultaneously. Since results secured by different workers have not always agreed, the seemingly best descriptions were used in compiling the table. Descriptions are available of other characters possessed by some of these bacteria (26, 68).

These nine gall-forming bacteria (Table 2) have the following characters in common, which are omitted from the table: all are small rods approaching one another in size, are not spore-formers and are not acid fast; many of them form chains under favorable

## TABLE 2

### COMPARISONS BETWEEN CHARACTERS OF VARIOUS CELL-STIMULATING BACTERIA

| ORGANISM | OXYGEN | | GELATIN LIQUEFACTION | NITRATE REDUCTION | CHROMOGENESIS | REACTION FROM CARBON SOURCES | | | | | | | | VEGETATIVE CELLS | | | | | MILK | | INDOL | HYDROGEN SULPHIDE | AMMONIA | THERMAL DEATH POINT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | DEXTROSE | | LACTOSE | | SUCROSE | | GLYCERINE | | | | | | | | | | | | |
| | AEROBE | FACULTATIVE ANAEROBE | | | | ACID | ALKALIN | ACID | ALKALIN | ACID | ALKALIN | ACID | ALKALIN | LENGTH (MICRONS) | DIAMETER (MICRONS) | CHAINS | GRAM | CAPSULES | CURD | PEPTONIZATION | | | | |
| PHYTOMONAS TUMEFACIENS (61, 52, 87) | + | | 0 | 0 | WHITE | T | 0 | T | 0 | 0 | + | + | 0 | .75-2.25 | 0.3-1.0 | | 0 | + | | | 0 | | + | 52°C. |
| PHYTOMONAS RHIZOGENES (61, 87) | + | | | 0 | WHITE | + | 0 | + | 0 | T | 0 | | | 0.5-2.6 | 0.1-0.8 | | 0 | + | | | 0 | | + | 52°C. |
| PHYTOMONAS SAVASTANOI (76, 52, 78) | + | | 0 | 0 | WHITE | + | 0 | 0 | + | 0 | + | T | 0 | 1.2-3.0 | 0.4-0.8 | + | 0 | | 0 | | + | | ? | 43°C. 46°C. |
| PHYTOMONAS TONELLIANA (75, 52) | + | | 0 | | WHITE | + | 0 | 0 | + | 0 | + | + | 0 | 1.5-2.5 | 0.5-0.6 | | | 0 | 0 | | + | | ? | 51°C. |
| PHYTOMONAS BETICOLA (9, 52) | + | | + | + | YELLOW | + | 0 | + | 0 | + | 0 | + | 0 | 0.6-2.0 | 0.4-0.8 | + | 0+ | + | + | | 0 | + | + | 51°C. 52°C. |
| PHYTOMONAS FASCIENS (91) | + | | 0 | + | YELLOW | + | 0 | 0 | 0 | + | 0 | + | 0 | 1.5-4.0 | 0.5-0.9 | + | + | ? | 0 | | 0 | + | + | 55°C. 57°C. |
| PHYTOMONAS GYPSOPHILAE (10) | + | + | + | + | WHITE, LATER YELLOW | + | 0 | 0 | 0 | + | 0 | + | 0 | 0.4-1.2 | 0.2-0.8 | + | 0 | + | + | + | 0 | T | + | 52°C. 53°C. |
| PHYTOMONAS RUBI (29, 52) | + | + WEAK | 0 | 0 | WHITE | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 1.72 | 0.64 | + | 0 | | + | | 0 | 0 | + | 56°C. |
| PHYTOMONAS PSEUDOTSUGAE (24) | + | + | + | + | WHITE | + | 0 | 0 | | 0 | | 0 | | 1.9-3.9 | 0.5-1.5 | | 0 | | | | | + | 0 | |

+ MEANS POSITIVE, 0 MEANS NEGATIVE, T MEANS TRACE

conditions. No gas was detected from nitrate and none with Smith tubes from fermented carbohydrate.

Bacterial organisms, other than those listed, sometimes have been described as gall-forming; *e.g.,* the root-nodule bacteria; *Pseudomonas pini* (16), an organism associated with a gall on pine trees; and undescribed bacteria (13) found in galls on certain forms of algae belong to the Florideae. The first of these are omitted from the list because root-nodule bacteria are beneficial rather than harmful, and the literature about them has been reviewed elsewhere (12, 20, 99). The evidence for pathogenicity of the pine-gall organism is incomplete (19), and no adequate description was found of the algal gall-formers.

The morphology of crown-gall bacteria has received special study, and a morphological irregularity has been observed in them (41, 43). In 48-hour-old cultures of the hop strain, there were Y-shaped cells and a few scattered coccoid bodies. Some of the cells had a beaded effect and others a capsule-like covering. In some cases the rods appeared to be arranged in irregular form, centering about small granular bodies. In 7- to 10-day-old cultures the rod fragments gave coccoid bodies and ghost cells. After 19 days the slime-like substance contained numerous coccoid bodies which were considered to be physiological spore-like bodies. Dry 90-day-old cultures, upon transfer to fresh media, resumed growth showing the characteristic rod-shaped forms. Others (69) have found no "Y" or warty forms. Irregular forms by *P. gypsophilae* (10), *P. beticola* (9) and *P. fasciens* (37, 91) have been observed.

A filterable stage in the life cycle has been suggested or claimed for several of these bacteria. This has been based upon experiments in which they were recovered from a filtrate. However, as Zinsser (101) has explained in detail, this does not constitute a filterable phase of a cycle. Since his postulates for demonstrating such a cycle have been fulfilled with none of these bacteria, such suggestions may be considered with reservation.

The common star-shaped aggregates in liquid media, like those from which *Radiobacter* derived its name, have suggested (85) fusion. This interesting concept deserves further study, especially with an electronic microscope and biochemical tests, to establish the character of the central granule and to rule out other possibilities, such as tangled flagellae or attraction by a particle with an electric charge different from that of the bacteria.

In physiological characters Table 2 shows various similarities and differences with perhaps one or two clues regarding pathogenicity. None of the bacteria was observed to produce gas from nitrate or from sugars without special study. With better technique all these cultures would probably produce some gas from sugars, as *P. tumefaciens* did (15). All produced some acid from dextrose, although at least with *P. tumefaciens* this probably came only from dissolved carbon dioxide. Ammonia production was of special interest because it was associated with the pathogenicity of *P. tumefaciens* almost three decades ago (77). Although present-day methods might question this early technique, there is no doubt that this and the hairy-root organism do produce ammonia (14, 97). Likewise all the other gall-inducing bacteria have been reported to produce ammonia except *P. savastanoi, P. tonelliana* and *P. pseudotsuga.* With the first two, litmus milk and certain peptone with sugar media developed an alkaline reaction suggesting ammonia production (75, 78). Since this is such a common character and notwithstanding the negative report, *P. pseudotsugae* would probably show it if studied with suitable technique. This comes near to being a common character associated with gall production.

There are other items which have been discussed (59) in relation to the pathogenicity of crown-gall bacteria, but concerning which much less is known in relation to the other gall-forming bacteria. For example: (*a*) *P. tumefaciens, P. rhizogenes* and *B. radiobacter* all reduced the oxidation-reduction potentials in several media containing plant extracts, suggesting that such bacteria growing in injured tissue might induce an "oxygen hunger". (*b*) The pathogenicity of crown-gall bacteria was destroyed by successive transfers in a dozen amino acids. Apparently this is the first instance where a pathogenic bacterium has been attenuated by a natural host constituent for which the chemical formula is well known. These studies have emphasized the importance of nitrogen metabolism in relation to pathogenesis. (*c*) All the gall bacteria apparently produce more or less gum-like material in culture. Such material from crown-gall bacteria has been identified as a polysaccharide containing approximately 22 anhydroglucose units per molecule. Such a diffusable and hygroscopic substance might easily disturb the osmotic relations of invaded tissue. (*d*) In addition to the ammonia produced by almost, if not all, gall bacteria,

*P. tumefaciens* produced phosphatides, phospholipids and other substances that might "irritate" neighboring cells. (*e*) In addition, it produced various enzymes, growth substances and vitamins, such as thiamin, riboflavin, pantothenic acid and biotin. These and other factors have influenced the distribution and availability of food materials.

An unusually promising working hypothesis has been developed as a result of these studies. There have been many explanations for the cause of pathological growth, depending on this or that item (reviewed, 62). However, the first working hypothesis found in either plant or animal literature that emphasizes the importance of a suitable balance between critical items was expressed (59) as follows:

"Among these factors, as we have already seen, may be included 'oxygen hunger', changes in osmotic pressure, rearranged amounts of growth substances and vitamins, 'irritating' substances, and altered amounts of food materials. Any living cell, even a resting cell, that fails to react under such conditions seems very unresponsive.

"While we shall continue to analyze individual factors that by their presence or absence may change normal into pathological growth, there is another possibility that deserves consideration. This is that in normal growth a number of factors may operate in suitable balance. However, in pathological growth of one kind a group of these factors may be out of balance. Likewise, in pathological growth of another kind the balance is disturbed in some other way".

The literature on the physiology of crown-gall bacteria extends far beyond that of other gall organisms, and so its consideration is omitted in favor of comparisons between host-parasite interactions.

## COMPARATIVE LIFE HISTORIES RE PATHOGENESIS

The pathological relations between host and parasite seem largely analogous in regard to entrance into the host, position in the host cells, exit from the host, and distribution to the new host. This approach is adapted from that devised (82) for work with animal pathogens. The situation is summarized in Table 3.

Entrance in almost every case reported in the foregoing tabulation was dependent on one or another type of wound, which varied

according to circumstances. For example, in olive knot the entrance was through leaf scars, which were most susceptible immediately after leaf fall. This mode of entrance was less and less favorable with progressing time, and the infection court was closed

TABLE 3

COMPARISONS OF SOME CRITICAL POINTS IN THE LIFE HISTORIES OF VARIOUS CELL-STIMULATING BACTERIA IN RELATION TO THEIR PATHOGENESIS[3]

| Causal organism and authorities | Entrance | Primary location | Exit | Distribution |
|---|---|---|---|---|
| *Phytomonas tumefaciens* (1, 54, 63) | Wounds | Intercellular | From surface | Nursery stock, insects, cultural operations |
| *Phytomonas rhizogenes* (28) | Wounds | Intercellular | From surface | Nursery stock, insects, cultural operations |
| *Phytomonas savastanoi* (27, 33, 76, 98) | Wounds, leaf scars | Intercellular | From surface | Nursery stock, pruners |
| *Phytomonas tonelliana* (75) | Wounds | Intercellular | ..... | Nursery stock, insects (?) |
| *Phytomonas beticola* (9, 17, 18) | Wounds | Intercellular | From bacterial pockets to surface | Debris from infected galls |
| *Phytomonas fasciens* (37, 38, 39, 91) | Not dependent on wounds | Intercellular | From surface | Seed-borne |
| *Phytomonas gypsophilae* (10) | Wounds | Intercellular, Intracellular in water-soaked areas | ..... | Nursery stock, soapwart weed |
| *Phytomonas rubi* (2, 29) | Wounds | Intercellular | From surface | Insects, pruners, cultural operations |
| *Phytomonas pseudotsugae* (24) | Wounds | Intercellular | ..... | Insects |

[3] Various important and sometimes modifying details are given in the text.

completely by the ninth day (27). Infection was also accomplished through natural fissures in the bark (33). Oleander was successfully inoculated by spraying the pistils (75). In crown gall on red raspberry, the bacteria entered through wounds to the roots caused sometimes by cultivation but more frequently by root-feeding

arthropods, such as click-beetle larvae, millepedes and white grubs (1). The type of wound apparently had little if any effect upon the character of the overgrowth, which was determined primarily by the species of infecting bacteria; but it did influence the percentage of wounds that became infected, the size of the infection and the rate of development. Various authors (30, 54) have found that the size of the wound influences the size of the developing crown-gall or hairy-root overgrowth. Although infection by fasciation bacteria on sweet peas was reported (37) not to be dependent on wounds, the possible activity of soil insects and the importance of wounds "that occurred incident to sprouting", as reported by Siegler and Bowman for peach (70), seems not to have been eliminated. The percentage of infection by hairy root through wounds made with a scalpel cut (28) was 71; with a bruise, 66; and with needle punctures, 41. Shallow wounds which did not penetrate to the cambium were less favorable infection courts than deeper wounds. Injuries made under ground commonly stayed open for three days but were closed after a week. The open infection court was maintained longer in moist soil than in dry soil. The wound did not need to be large. Experimentally (30, 54) tiny needles about 30 microns in diameter could open infection courts in tomato. In some cases (30) a single motile bacterium, introduced with a micromanipulator, induced infection. A high percentage of disease was secured when 100 or more bacteria were employed. Very small galls on tomato stems were induced by ". . . gently stroking the stems and petioles of tomato plants with a . . . needle previously moistened" with a suspension of bacteria (30). Usually these galls never attained a size greater than that of one or two millimeters. Just where the bacteria were introduced in these cases and why the galls never became larger have not been clarified.

Location of the bacteria in the host and their movement through the tissue are such large topics involving "secondary galls" and "tumor strands" that they are postponed until after consideration of exit and distribution.

The exit of gall bacteria (2, 10, 27, 28) from diseased tissue has seemed to be from the surface. In certain cases the bacteria have occurred not only in gelatinous material on the surface but also between the cells or in pockets that were composed of dead and disintegrating cells. With the interior growth of the galls or by

other means the bacteria reached the surface from which they could be removed by water, as summarized in Table 3. The evidence (2), especially regarding cane gall, was of four types: (*a*) many cavities obviously created by bacteria near the surface held only a few or no bacteria, (*b*) bacteria were always found on the surface, (*c*) continuous escape of the bacteria has been demonstrated experimentally, and (*d*) bacteria have been observed in the process of discharge via intercellular channels.

Disintegration of galls has seemed an obvious means for releasing the bacteria. However, the large number of active secondary organisms present has made difficult the isolation of pathogenic bacteria. There was even a question whether some of the gall-formers could survive the competition.

Dissemination of the various gall organisms occurs in a variety of ways common to the dispersal of plant pathogens. Since over half of the organisms live naturally on trees and bushes, a very common means of spread apparently is with nursery stock. Several writers (*e.g.*, 28) have found that *P. tumefaciens* and *P. rhizogenes* lived in the soil for over a year, implying that if nursery plots became infected they were quite likely to remain so for some time. Although much longer periods have been recorded, the bacteria may have been reintroduced by running water or other means. Insects have been reported by various men (*e.g.*, 21, 24, 75) to carry the bacteria from tree to tree and to allow entrance through feeding or ovipositing wounds. Local dissemination by rain-washed and wind-blown droplets has been important, as in the case of *P. savastanoi* (27, 98). *P. fasciens* was seed-borne (91). Weed hosts might act as the source of infection; *e.g.*, *Rumex acetosella* provided (51) crown-gall infection for beets. Soapwort weed was mentioned (10) as a host to *P. gypsophilae*.

### LOCATIONS OF BACTERIA WITHIN THE HOSTS

Location of the bacteria in host tissue is similar for different bacteria, as might be expected from the manner in which they gain entrance through wounds. When a wound is made, the liquid from injured cells moves into the neighboring intercellular spaces and provides a direct liquid channel for the bacteria. Various details of this aspect have been worked out (2, 28, 54, 67) for crown-gall, cane-gall and hairy-root bacteria, which may apply also to other gall bacteria.

The intercellular location of crown-gall bacteria, although questioned (40, 42, 50), has been based upon the following lines of evidence: (*a*) The galls developed in the areas where liquid from wounds entered the intercellular spaces (54). (*b*) Galls developed from flooded areas when the region of the original wound was killed by heat (54). (*c*) There was a correlation between the size of the wound (30, 54), the corresponding flooding of intercellular spaces (54) and the size of the galls which developed. (*d*) The bacteria have been observed in the intercellular spaces (*e.g.*, 2, 3, 22, 32, 46, 54, 55, 67), and were often surrounded by rapidly dividing cells (54, 67). (*e*) Translocation of the bacteria and of inert material, such as carbon in suspension, has been observed through the intercellular spaces (34). (*f*) When bacteria entered a wounded cell and grew, the cell died (54). (*g*) When small numbers of bacteria were injected with a micropipette inside cells, the bacteria ordinarily died. If they survived, no division of the including cell took place (30). (*h*) The bacteria, seen between cells in living uncontaminated sections placed in agar, have been observed to grow, have been isolated and have been identified (54).

Questions regarding their locations have appeared from time to time because of difficulty in staining and in interpreting the results with a good stain. With a satisfactory section the bacteria have often been seen in an intercellular space. Sometimes they were distributed through a partly dissolved middle lamella. When such a wall was viewed from the edge, their intercellular position was clear. However, when the thin wall was viewed from the flat side, it sometimes appeared as if the bacteria were inside the cells above or below.

Various materials both inside and between the cells that might be confused with bacteria have received special attention with both crown gall and cane gall (2, 54). These materials included tannin, crystals, starch, fat globules, pectic granules, mitochondria, chondriosomes, young plastids and various other cell inclusions. Special techniques have been employed for determining the nature of each of the various items. Banfield concluded that ". . . the bacteria-like bodies observed by Smith and later by Pinoy and Nemec within tissue cells of crown galls were normal elements of the chondriome of the cells and not bacteria as they at one time believed. . . ." Five lines of evidence for this conclusion were given.

An intracellular position of crown-gall bacteria has been observed occasionally (46, 56) in wounded cells or old cells that were no longer dividing. In galls on plum the "bacteria" were observed (50) often arranged in threads inside the cells and never between the cells. However, normal cell elements may have been mistaken (2) for bacteria. If substantiated, this might be compared with the situation worked out for the legume root nodule bacteria. Intracellular positions have been observed with other gall-forming bacteria (10, 38).

As the gall cells developed for a few weeks, conspicuous swelling and multiplication of cells were found (54) about the position of the bacteria, which often indicated their location. In later stages the progression of cellular proliferation seemed like "appositional" growth (53, 79).

### MOVEMENT OF BACTERIA IN TISSUE

Development of galls is influenced, doubtless, by changing bacterial locations. The manner in which bacteria move along with the liquid released by a wound and thus invade the intercellular spaces has already been discussed. Further enlargement of a gall as it involves more and more tissue has been partly explained (79) thus: as the gall develops, some of the cells are crushed, which releases the cell contents to flood still more intercellular spaces and to provide channels for further bacterial movement and activity. Under certain natural and experimental conditions, flooding of the tissues might extend for some distance. Somewhat related flooding of tissue has been of well known economic importance, for example, in water core of apples and pears, in the internal breakdown of celery, and in favoring infection of tobacco by several bacteria (35). It has been observed on many plants following rain and lowered temperatures.

Bacterial movement in artificially water-soaked tissue (54) was found throughout the 10 cm. of tomato stem that were flooded, indicating that the limit had not been reached. If the wound occurred under suitable conditions near the apex of a condensed bud, like that of a sunflower, it was possible for the liquid from injured cells to flood the intercellular spaces past a number of internodes and for the infecting bacteria to follow. Subsequent elongation of these internodes separated different portions of the infection by con-

siderable distances. This separation was later shown by "tumor strands" and "secondary galls" (*e.g.*, 55, 67). Water-soaked areas have also been observed in other galls (*e.g., Gypsophila* galls, 10). In addition to the liqiud from wounds and from cells crushed by growth of nearby tissue, the flooding of air spaces by several physiological means has been found relatively common. When flooding occurs from wounds in connection with bacteria or from vascular elements, one has perhaps the easiest explanation for further distribution of the bacteria.

Similar "secondary galls" in beets have occurred (17) after the bacteria entered the tracheids from colonies in pockets, traveled in the transpiration stream and broke out into new pockets. In hairy-root the presence of bacteria in vessels induced no changes in the surrounding tissues and was considered of no importance (28). In oleander knot "secondary galls" appeared up from the original infection and less often down from it. The travel was observed in stems (75) to be through definite channels of infection developed in the actively growing succulent tissue. These channels were often arranged in nearly straight lines and passed several internodes. "In the leaves the channels of infection apparently follow the veins but are apparently distinct from the vascular system, as cross-sections of veins and petioles fail to show the organism present in the vessels, while masses of bacteria are readily found in the parenchyma". In olive knot the bacteria moved through the vascular system to form "secondary galls" (27, 76). The bacteria accumulated in the ends of vessels (27) and seemed not to break out unless they were released into other tissues of the leaf scar when the leaf fell. With sunflowers grown in the greenhouse, "secondary galls" were found (4) developing at leaf scars.

The passage of virulent crown-gall cultures through vascular tissue has been rapid and extensive. In distances from point of entry cultures have been isolated at 8 cm. in fruit trees (86), 15 cm. in tomato (88) and 120 cm. in *Datura tátula* (86). The senior writer (unpublished) has found the bacteria in vessels of apple as far as the vessels were open. It has been suggested (88) that the accumulated bacteria in the vessels of tomato stimulated the formation of adventitious roots. The possibility that the bacteria then moved through the vessels of these roots to form "secondary tumors" (88) was suggested. With a similar idea regard-

ing "secondary tumors", Braun said (4): "While one may not feel fully confident of the exact course followed by the bacteria. . . . It is believed probable . . . that the bacteria remain confined to the vessels and that under their influence cell-stimulating substances are formed that diffuse laterally and bring about cellular disturbances in adjacent tissues". These interesting concepts may be considered with reservation in view of three lines of evidence: (*a*) The bacteria have been present in vessels without causing galls (*e.g.*, 54) until they were released into the surrounding tissue. (*b*) The bacteria have been in contact with several kinds of uninjured living cells for long periods without causing galls (60, 65). (*c*) The formation of "secondary galls" is explained in other ways.

The results that Braun (4) secured may be clarified perhaps by the pictures of his experimental plants. These appeared long and spindly, like short-day and high-temperature sunflowers, as they developed during cold weather in the greenhouse. Such succulent plants in Wisconsin have had the air spaces of the stems flooded frequently. The internal stem pressures have sometimes been great enough even in uninoculated plants to split open the stems. Consequently inoculations made four and six inches below the growing tip might have the benefit of a continuous liquid channel for some inches both up and down. In this case the bacteria could invade the condensed bud and be carried upward still further by elongation of the subsequently developing internodes. Since Braun's photographs look like such plants, perhaps his results could be explained by various earlier reports (*e.g.*, 54, 55, 67). The question might be raised whether he always differentiated between parasitic and non-parasitic galls, discussed later, because (*a*) he secured "secondary galls" with attenuated cultures and without a primary gall, (*b*) in his illustrations these galls appeared quite small, and (*c*) he was able to isolate crown-gall bacteria from them in only a few instances. However, this last was not surprising in view of the frequent reports that the bacteria were not isolated even from primary galls. These "secondary galls" on sunflower in which the bacteria were dead, ordinarily have failed at Wisconsin to develop further unless they were taken out of the parent plant and placed on tissue culture media (31). Even in such cases the writers have experienced a high percentage of fail-

ures. Cultures of tissue, in which active growth was started by crown-gall bacteria and was continued after the bacteria were no longer present, are listed later with non-parasitic developments.

Elongated groups of proliferating cells, called "tumor strands", have frequently been found in regions between "primary" and "secondary" galls. There seems to be general agreement in the last 25 years that these strands sometimes fail to connect the two kinds of galls. Consequently the "secondary gall" does not develop as an outgrowth or branch of the primary gall. The "strands" probably grow about the narrow channels through which the bacteria pass in flooded intercellular spaces. In some cases these channels (55, 67) have been elongated as a result of expansion by condensed buds.

The distributions of bacteria by means of liquid within intercellular spaces, of elongated growing tips, and of release from injured vessels carrying bacteria permit analogies between the "secondary galls" formed by crown gall, olive knot and other galls.

The "secondary galls" on sunflower from which the bacteria could not be cultured have provided excellent material for tissue culture isolations (6, 96). When grafted back into sunflower this callus-like tissue continued to grow. Similar cultures have been secured (94) from non-parasitic galls on tobacco and other plants which are considered later.

The further extensive physiological literature about crown gall and its causal agent is here omitted because of its volume and because there is practically no comparative information about the other bacterial diseases. However, a brief mention seems appropriate of some related developments not caused by micro-organisms.

## NON-PARASITIC GROWTHS

A variety of non-parasitic but pathological growths have been described which in some cases have been confused with the bacterial galls. Wound overgrowths on piece-root-grafted apple trees, for instance, were for a long time called crown galls. However, they have been differentiated from bacterial galls and their identity as a non-parasitic difficulty proved (65). They resemble the overgrowths induced by a wire girdle and by a cut made part way through the stem (63). In such cases the enlargement is associated with an accumulation of food materials as they move downward.

A closely related enlargement has frequently occurred at the union between a vigorously growing scion and a dwarfed root stock (57). Although relatively rare in the United States, it has been found frequently in Europe where dwarfed apple trees are commonly propagated.

Bur knots were considered at one time to be the result of crown-gall infection, but more recently (8, 89) they have been removed from the category of bacterial diseases. They have appeared with great frequency on seedling apple trees, and many such seedling trees have been considered undesirable because of this character. The bur knots consist primarily of clusters of root initials which appear especially near the buds on the above-ground stems. As such stems are placed in the ground, the root initials grow and permit a new apple tree to develop from this cutting. It is these bur knots which enable the Doucin, Paradise and East Malling root stocks to serve as understock for known varieties.

Non-parasitic galls have also been observed by various workers (*e.g.*, 93) on the tobacco cross *Nicotiana glauca* Grah. ♀ × *N. langsdorfii* Wein. ♂. They were covered by an abundant growth of epidermal hairs and gave rise to shoots. The disorganized tissue was primarily parenchymatous with scattered vascular elements. It contained considerable starch and tannin. When isolated aseptically with procambial strands, unlimited growth has been secured of a white callus-like growth (94). When such tissue was placed under 8 mm. of liquid, stem growing points were differentiated which developed into short stems and formed leaves. The effect was attributed to reduced oxygen supply. However, differentiation in aqueous medium was completely prevented by indole-3-acetic or naphthaleneacetic acid which led to the conclusion (71): "Under certain circumstances the oxygen gradient is an important external factor operating to prevent organ formation, but its effect must be very indirect".

Isolations and cultivation *in vitro* have also been made (reviewed, 95), for example, from roots (tomato, sunflower, radish, clover, mustard, buckwheat, pea, flax, vetch, wheat), cambium (willow, poplar, oak, beech, pine, carrot), procambium (tobacco, squash, sunflower, potato tubers, kohlrabi), and various stem tips and embryos.

Various other non-parasitic growths have been induced by chemi-

cals (77). Among the most active have been indole-3-acetic acid, naphthaleneacetamide, and many others among the plant hormones. Galls induced by hormones have closely resembled crown gall (11, 36) and continued growth for some months. Decapitated bean plants treated with 3% indole-3-acetic acid in lanolin developed galls 2 cm. in diameter (23), mostly by growth from the pith. Irregular lateral outgrowths developed mainly from phloem derivatives. The tissues stimulated varied with different plants and different chemicals. Culture of tissue *in vitro* from galls caused by indole-oxalo-acetic acid showed (96) low growth rate and "a high degree of morphogenic conformity . . . ". Placed back in the same plant species such tissues failed to grow.

When such galls were induced by chemicals above the point of inoculation with attenuated bacteria, the tissue about this attenuated culture was stimulated as much as that about a virulent culture (5, 59). A similar phenomenon had been earlier observed when inoculations with a virulent culture were made above those with attenuated cultures (44). In some experiments (5) the inoculations and chemical treatments were so close that the bacterial or chemical cause was not too clear. The results were interpreted as follows: The "growth substances used . . . served merely to stimulate cells previously altered by the attenuated culture". When a longer distance between chemical and inoculation was employed (59), the same phenomenon was observed. Riker (59) concluded: "While one might jump to the conclusion that such a chemical is the factor missing from the region of the attenuated culture, caution is indicated because these chemicals induce considerable cell growth near the point of application. So these rapidly growing host cells may be providing the missing factor".

## SUMMARY

Comparisons have been made of nine bacterial plant gall diseases, *viz.*, beet pocket rot, cane gall, crown gall, Douglas fir gall, *Gypsophila* gall, hairy root, oleander knot, olive knot and pea fasciation, as well as some similar non-parasitic galls.

Some of these galls have been known for centuries. Several have been reported from widely scattered regions where they may have been distributed with nursery stock. Their economic importance has been great in some cases and small in others.

Scientifically they are of particular interest because they provide an opportunity for studying various aspects of pathological growth. The fundamental similarity between plant and animal cells, and the relative ease with which plant cells can be studied, have suggested that a clarification of diseased growth in plants would be helpful also in an understanding of similar conditions in animals and human beings. The word "cancer" has been avoided.

Among the factors encouraging fundamental work with plants are large numbers, low cost, suitable range of types including some resistant and others susceptible, easy experimental manipulation, pathological growths easily induced by micro-organisms and by non-parasitic agencies, genetic purity through pure lines or vegetative propagation, and cultivation *in vitro* on nutrients with known chemical formulae.

Susceptible and resistant hosts and virulent and attenuated pathogens as well as host specificity have been observed.

Crown gall has appeared on many hosts; the other bacterial galls on relatively few. Investigations of crown gall and its causal agent have been extensive; those of the other diseases rather limited.

Among the bacteria many morphological and physiological similarities and differences have been listed. Production of ammonia by almost all, if not all, of these organisms is noteworthy.

A working hypothesis for the initiation of pathological growth has suggested a disturbed balance between various critical factors, including perhaps oxygen tension, nitrogen metabolism, osmotic relations, "irritating" metabolic products, food materials and growth substances.

Entrance by gall bacteria into the host is usually through wounds; their exit is apparently from the surface of living galls. Dissemination occurs in various common ways.

The stimulating bacteria inside tissues have been located between the host cells. They have sometimes been found inside cells that seemed no longer active. Although the position of the bacteria has been occasionally questioned, much evidence has accumulated in favor of an intercellular position.

Various bacteria progress through the tissue in several ways and form "secondary" galls as well as "tumor strands". For example, they move in intercellular spaces flooded by the liquid from injured cells or from physiological disturbances. In the latter case they

sometimes move several inches. When the liquid containing bacteria involves a condensed bud, subsequent elongation of the internodes provides still further separation. The bacteria also travel with the open sap stream after entry through injured vessels, and form "secondary galls" when released into the surrounding tissue.

Strands of pathological tissue often extend from "primary" to "secondary galls", but there was no actual connection in a number of cases.

More or less closely related to bacterial galls are various nonparasitic galls caused, for example, by wounds, grafts, accumulated food materials, genetic characteristics and certain chemicals including "plant hormones". Callus growths *in vitro* and their grafts back into the host are noteworthy.

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